gene in 13 % cases. It was found that FAD genes mutants affect calcium homeostasis in hippocampal neurons by disrupting Ca^{2+} storage in the lumen of endoplasmic reticulum (ER). In our study we used electrophysiological recordings with a patch-clamp technique in whole-cell mode and calcium imaging with Fura-2AM to study calcium channels activity, ER calcium load and ER calcium leak. We observed different effects of FAD mutated genes expressions on activity of store-operated and L-type voltage-gated calcium channels in mouse hippocampal neurons, mouse neuroblastoma Neuro2a and human neuroblastoma SK-N-SH cell lines. We found that effects of FAD PS1 mutants on calcium channels were driven by misbalance in activity of ER calcium sensors STIM1 and STIM2. The different effects were connected with calcium sensors STIM1 or STIM2 impaired signal transduction from ER to calcium channels in plasmatic membrane (PM) under control of ER calcium levels. The impaired signal transduction was revealed in live confocal imaging experiments. The enzyme activity of PS1 was involved in regulation of store-operated calcium entry in two ways: the activity of channels was depended on PS1 endoproteolysis level and APP cleavage.

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2777-Pos Board B469

Tau Protein Forms Ion Channels

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Tau is a microtubule associated protein that is intrinsically disordered and accumulates in amyloid fibril deposits alone and in combination with other amyloid forming proteins. We have found that Tau 441 can form ion- permeable channels in planar lipid bilayers under conditions of acidic pH. The channels are irreversibly associated with the membrane and exhibit heterodisperse conductances. Acidic phospholipids enhanced channel activity. The channels are only weakly selective for cations. Zn^{++} does not block the channels. These channels show significant, but incomplete, similarity to the amyloid channels formed by the Alzheimer peptide Abeta, the diabetes associated peptide IAPP, the prion related PrP106-126 ,and other amyloid channel peptides. Channel forming activity may play a pathogenic role in Tau associated diseases such as Lewy Body Dementia, by causing ion leakage in target cell membranes.

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Alterations in Ionic Currents and Gap Junctional Coupling by Pan-Histone Deacetylase Inhibition

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Dynamic balance of histone acetyl transferase and histone deacetylases (HDACs)is maintained in multiple signaling pathways determining expression and repression of genes. Pan-HDAC inhibitors like Vorinostat(VOR, ZolinzaTM) and Romidepsin (Istodax(r)) have been approved for treatment of peripheral and cutaneous T-cell lymphomas. On rare occasions during clinical trials,HDACi has been associated with symptomatic ECG alterations, cardiac arrhythmias, and even sudden cardiac death for unknown reasons. Rationale of this study is to identify electrophysiological and molecular determinants involved in asymptomatic cardiac abnormalities in patients treated with HDACi during clinical trials. We investigated the effects of VOR on ionic currents and gap junctions responsible for cardiac excitability and propagation. Cultured neonatal mouse ventricular myocytes (NMVM) were treated with 1-5μM VOR overnight and patch clamp experiments were performed to measure the transient (Ito) and steady state (IK) outward K^+, peak inward Na^+ (INa), and peak inward L-type Ca^{2+} (ICa,L) current densities. Dose-dependent decreases in INa density and NaV1.5 protein levels occurred along with +10mV shift in INa activation in myocytes treated with 1-5μM VOR. Conversely, ICa,L density increased significantly without concomitant change in CaV1.2 protein expression levels. Moreover, augmentation of ICa,L byVOR was independent of stimulation with 5μM isoproterenol, suggesting discrete acetylation and phosphorylation dependent mechanisms for modulation of cardiac ICa,L However, VOR did not alter any IK or Ito current densities. Also, VOR dose dependently reduced gap junction coupling and protein expression of connexin43. Pan-HDACi treatment significantly reduces Cx43 protein expression and coupling along with salient alterations in INa and ICa,L densities. These ion channel changes present possible etiologies for cardiac abnormalities in patients. VOR being a pan-HDAC inhibitor, we plan to determine the effects of isoform-selective HDACi on ionic currents and APD in human cardiomyocytes derived from induced pluripotent stem cells.

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Self-Assembly of the Viral Channel Forming Protein Vpu of Hiv-1 using Coarse-Graining Molecular Dynamics Simulations

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Vpu of human immunodeficiency virus type 1 (HIV-1) which is an 81 amino acid bitopic membrane protein known to homo-oligomerize. It is known for its ability to form channels and to assemble into host factors. The assembly process and the oligomer state of Vpu are not known. Coarse-graining molecular dynamics simulations are performed with full-length Vpu in lipid bilayers. Full-length Vpu is constructed by linking an experimentally derived structural model of the cytoplasm domain with a computationally generated transmembrane domain (TMD). Simulation boxes are generated harboring selected numbers of VPUs. The simulations identify that Vpu assembles via several binding sites into a series of oligomeric states. Most striking, in a simulation of four Vpu's the proteins form a symmetric bundle which is a close match to known channel architectures. The tetramer is followed a dimer formation. Not only TMDs but also cytoplasm domains are involved in the dynamics of self-assembly.

2780-Pos Board B472

Ion-Trapping in HCV P7 Hexameric Bundles - A Molecular Dynamics Simulation Study

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Viral channel forming proteins assemble into homo-oligomers when produced within the infected cells and full fill their role as diffusion-amplifier for ions across subcellular membranes. Alteration of the electrochemical gradients seems to be necessary and in some cases essential for the survival of the virus. Much is known about the structural features of many of the channels, dynamics data about oligomerization, assembly and ion diffusion within the assembled bundles is still lacking.

The dynamics of physiological relevant ions, Na^+, K^+, Cl- and Ca^{2+} are monitored in the vicinity of hexameric bundles of p7 of Hepatitis C virus (HCV), strain 1a. The bundles are generated by a combination of docking approach and molecular dynamics simulations. Ion dynamics is recorded during multi 200 ns MD simulations of 1 M solutions. With a crucial residue, histidine-17, found to point into the lumen of the pore, protonation of this residue is altered and the effect on the ion dynamics monitored. While an 'unprotonated' p7 bundle allows Ca^{2+} to enter the lumen, it is exclusively the Cl- in case of a 'protonated' bundle.

2781-Pos Board B473

Structure and Inhibition of the M2 Proton Channel from the Influenza a Virus

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The influenza A virus M2 proton channel (A/M2) is the target of the antiviral drugs, amantadine and rimantadine, whose use have been discontinued due to widespread drug resistance. Among the handful of drug-resistant mutants of M2, S31N, V27A and L26F were found in more than 99% of the currently circulating viruses. Discovery of inhibitors targeting these M2 mutants has been hampered by the lack of structural information and their limited sizes, polarity, and dynamic nature of their drug binding sites. Nevertheless, using an integrated approach including medicinal chemistry, molecular dynamics simulation, solid/solution-state NMR, X-ray crystallography, and pharmacological characterizations, we have discovered small molecule drugs that inhibit mutant M2 (S31N, V27A and L26F) with potencies greater than amantadine's potency against WT M2. A few compounds exhibiting EC50 around 100 nM are advanced to mice model studies. Structural characterization of S31N drug binding by NMR shows the drug bound in the homotetrameric channel, threaded between the side chains of Asn31. The S31N inhibitors, like other potent WT M2 inhibitors, contain a charged ammonium group. The ammonium binds as a hydrate to one of three sites aligned along the central cavity that appear to be hotspots for inhibition. These drug binding hotspots along the channel axis provide a general model of M2 inhibition that can be used to guide the design of other channel blockers.

2782-Pos Board B474

Mechano-Sensitive Ion Channels (MSCS) Provide Human Breast Cancer Cells with a Sensorium for Mechanical Stress

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Mechanical stress is increasingly recognized as a cancerogenic factor in breast cancer. We investigated the biophysical characteristics of mechano-sensitive